Correlates of broadly neutralizing antibody development

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Abstract: PURPOSE OF REVIEW Broadly neutralizing antibodies (bnAbs) are considered a key component of an effective HIV-1 vaccine, but despite intensive efforts, induction of bnAbs by vaccination has thus far not been possible. Potent bnAb activity is rare in natural infection and a deeper understanding of factors that promote or limit bnAb evolution is critical to guide bnAb vaccine development. This review reflects on recent key discoveries on correlates of bnAb development and discusses what further insights are needed to move forward. RECENT FINDINGS An increasing number of parameters have been implicated to influence bnAb development in natural infection. Most recent findings highlight a range of immune factors linked with bnAb evolution. Novel approaches have brought exciting progress in defining signatures of the viral envelope associated with bnAb activity. SUMMARY Focused efforts of recent years have unraveled a multiply layered process of HIV-1 bnAb development. As it is understood today, bnAb evolution can be triggered and influenced by a range of factors and several different pathways may exist how bnAb induction and maturation can occur. To capitalize on the gained knowledge, future research needs to validate factors to identify independent drivers of bnAb induction to advance vaccine design.

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Correlates of broadly neutralizing antibody development

Irene A. Abela[a], *Claus Kadelka*[b], *and Alexandra Trkola*[a]

Purpose of review
Broadly neutralizi''ng antibodies (bnAbs) are considered a key component of an effective HIV-1 vaccine, but despite intensive efforts, induction of bnAbs by vaccination has thus far not been possible. Potent bnAb activity is rare in natural infection and a deeper understanding of factors that promote or limit bnAb evolution is critical to guide bnAb vaccine development. This review reflects on recent key discoveries on correlates of bnAb development and discusses what further insights are needed to move forward.

Recent findings
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Summary
Focused efforts of recent years have unraveled a multiply layered process of HIV-1 bnAb development. As it is understood today, bnAb evolution can be triggered and influenced by a range of factors and several different pathways may exist how bnAb induction and maturation can occur. To capitalize on the gained knowledge, future research needs to validate factors to identify independent drivers of bnAb induction to advance vaccine design.

Keywords
broadly neutralizing antibodies, HIV-1, vaccine

INTRODUCTION

Neutralizing antibodies are a critical component of licensed antiviral vaccines [1], but despite intensive efforts, attempts to create an HIV-1 vaccine that induces protective antibody responses failed thus far. Neutralizing antibodies in natural HIV-1 infection are subject to rapid virus escape and predominantly target the autologous virus with limited cross-neutralization activity [2,3]. The discovery of broadly neutralizing antibodies (bnAbs) that neutralize close to all HIV-1 strains across diverse subtypes has revived HIV-1 vaccine development in the past decade [4,5]. Today, bnAbs are at the center of novel approaches considered in HIV-1 prevention, therapy, cure, and vaccine development [6–9]. Passive immunization with bnAbs in animal models of chronic HIV-1 infection and during analytic treatment interruption (ATI) of HIV-1 infected individuals has underlined the potency of bnAbs [10–13]. bnAb treatment of HIV-1 infection has shown the capacity to delay rebound during ATI, to decrease viremia and virus set points in a notable number of participants [10–12,14–16]. These capacities highlight prospects for bnAb-based therapeutics [17–20]. Importantly, bnAbs prevent infection in animal models by mucosal challenge [8,21–25] paving the way for bnAb-based vaccines. Numerous vaccine strategies are currently pursued and tested in animal models [26,27], and large-scale human studies are underway that probe the capacity of passively transferred bnAbs in preventing HIV-1 infection [28].

Despite many achievements in HIV vaccine development, the ultimate goal, induction of bnAbs by vaccination, has not been possible [5]. This has called for intensive research efforts aiming to understand how bnAbs develop in natural HIV-1

[a]Institute of Medical Virology, University of Zurich, Zurich, Switzerland and [b]Department of Mathematics, Iowa State University, Ames, Iowa, USA

Correspondence to Alexandra Trkola, PhD, Institute of Medical Virology, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland. Tel: +41 44 634 53 80; e-mail: trkola.alexandra@virology.uzh.ch

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KEY POINTS

- Define and validate factors that are linked with bnAb induction.
- Define surrogate markers of bnAb induction to allow early assessment of vaccine responses.
- Define Env signatures that promote bnAb induction.

infection. Induction of potent and broad neutralizing antibodies in HIV-1 infection is rare [29]. Knowledge of the factors that restrict or promote bnAb induction may thus provide crucial insight how the limitations in evoking these responses by vaccination can be overcome. In recent years, a rapidly increasing number of factors have been implicated as determinants in the development of neutralization breadth in natural infection (Fig. 1) but only a fraction has been confirmed across different cohorts as independently acting parameters [29–37].

Here, we report on current knowledge of the determinants of bnAb induction with a specific emphasis on the most recent reports, open questions, and goals for future research.

INFLUENCE OF HIV-1 INFECTION ON BROADLY NEUTRALIZING ANTIBODIES DEVELOPMENT

bnAb activity arises predominantly in viremic individuals after several years of infection [29,34,36,39] suggesting that prolonged exposure to viral antigen is needed for bnAb induction. Not only the duration of antigen exposure but also the amount of antigen appears to play a role. A factor most frequently found to positively influence neutralization breadth across all cohorts is high viral load [29–37,40,41]. Nevertheless, although rates of bnAb activity are significantly higher amongst individuals with high viral set points, breadth can also develop (albeit at a lower frequency) in HIV-1 controllers [31,40,42–45]. Hence, high antigenic burden promotes bnAb evolution but certainly is not a strict requirement.

Continued replication drives HIV-1 diversity and this feature has been confirmed as independent driver of bnAb induction [29,32,34–36]. Diversity of envelope (Env) is particularly high reflecting the effects of consecutive rounds of neutralizing antibody (nAb) attack, virus escape, and nAb maturation [46]. Studies deciphering the ontology of bnAb evolution in individual donors have highlighted the effects of this iterative process of bnAb maturation and viral escape that gives rise to Env variants that engage novel antibody germ lines and start a bnAb lineage [47,48]. In which contexts Env diversity is promoting bnAb evolution has however not conclusively been defined. Although several studies observed a positive effect [49] and some note links between bnAb evolution and superinfection with heterologous strains [39,50▪▪,51–53], the effect of superinfection is not consistently reported across cohorts and investigated cases [40,54▪▪,55,56]. A recent report by Sheward *et al.* [54▪▪] shows that consecutive exposure to heterologous Envs during superinfection leads predominantly to de novo antibody responses. Although this still could lead to a cumulative increase in polyclonal neutralization activity as shown by others [57], the authors observed no evolution of single bnAb specificities linked with the investigated superinfection cases ($N = 4$).

Only for few individual bnAb donors epitope specificity of the isolated bnAbs is known. The majority of analyses available to date rely on data of patient plasma for which bnAb specificity is either inferred on the basis of their neutralization

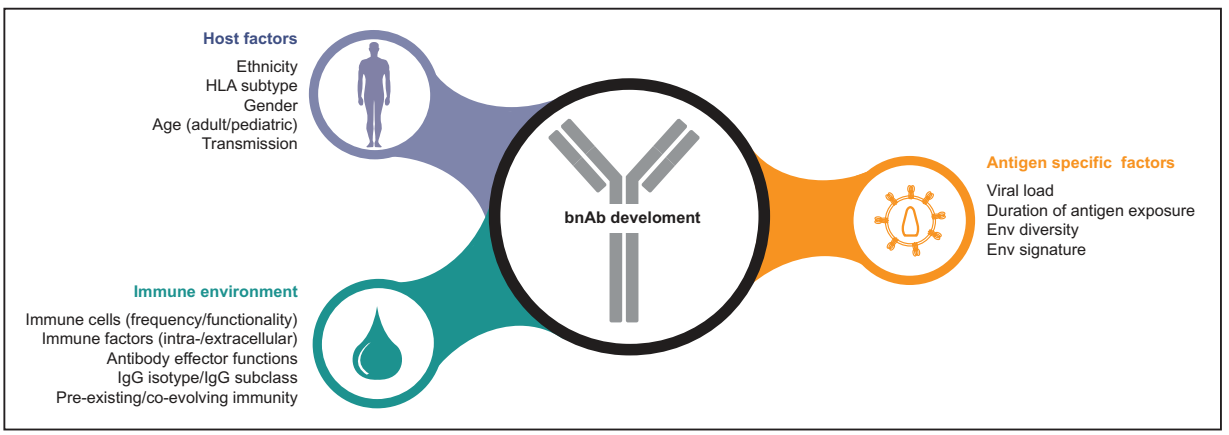

FIGURE 1. Determinants of bnAb induction suggested by studies reviewed here and in Subbaraman *et al.* [38].

fingerprint (referred to commonly as delineation of bnAb specificity) or not known. The impact of specific factors may thus also vary depending on the epitope specificity of the elicited bnAb. We found evidence that this may be the case for virus diversity in a recent study by Kadelka *et al.* [58▪▪]. Analyzing the Swiss 4.5K study for influence on neutralization breadth [29] ($N = 4484$) and binding antibody responses ($N = 4281$) [58▪▪], we defined diversity as an independent driver of neutralization breadth. However, when analyzed on the level of binding antibodies, diversity proved to only promote gp120 responses and not gp41 membrane proximal external region (MPER) antibody responses. Hence, crucial epitope-dependent differences may exist. It is plausible that antibody responses are not impacted by overall Env diversity if the targeted epitope region is highly conserved as it is the case for the MPER. In consequence, this also effects the interpretation of plasma neutralization breadth data. Differential influence of factors, such as evident for diversity, will remain difficult to disentangle as long as we cannot fully delineate plasma neutralization activity (including those with modest breadth) and distinguish between breadth that stems from additive polyclonal responses and breadth that stems from true bnAb lineages.

DEFINING Env SIGNATURES THAT DRIVE BROADLY NEUTRALIZING ANTIBODIES EVOLUTION

Evidence is accumulating that qualitative differences amongst Env variants exist that strongly impact and shape the bnAb response [29,59,60▪▪]. Genetic subtypes of HIV-1 appear to differentially steer bnAb responses. Potent $CD4^+$-binding site-targeting bnAbs were reported to more frequently emerge in subtype B infection [29,59], whereas V2-specific bnAb responses emerge predominantly in non-B infection [29]. Hence, Envs from different subtypes must carry distinct signatures that promote one bnAb specificity over the other that need to be defined. However, even within these subtypes, antibody responses vary strongly and different bnAb specificities emerge [29], highlighting the need to define more distinct signatures that drive bnAb development (see Doria-Rose *et al.*, in this issue). The overall Env diversity within and across patients and subtypes, paired with the comparatively low number of cases from which bnAbs have been isolated and characterized, has thus far limited the possibilities to define Env signatures that drive bnAb evolution. Although a number of Env motifs, glycosylation patterns, and variable loop length have been implicated to be linked with breadth induction and/or formation of specific bnAb specificities [61▪,62–65], transforming these into immunogens that evoke bnAb responses was thus far not successful. Considering the various distinct epitopes of the identified bnAbs types, each bnAb specificity may have its individual requirements on epitope composition and exposure on the immunogen. Deciphering Env signatures of different bnAb types will remain challenging but recently established, novel approaches to delineate resistance patterns of bnAbs are an important step into this direction. Application of machine-learning techniques [66,67▪▪] and mutational scanning and mapping [68▪,69▪▪,70▪] have brought an unprecedented level of detail to bnAb–Env signature analysis. In addition, novel approaches to delineate neutralizing plasma antibody responses have been developed. Advanced computational strategies to dissect polyclonal neutralizing antibody activity [71▪,72], epitope signature analysis of plasma responses by conventional binding assays [73▪], and advanced epitope mapping of polyclonal antibody responses by electron microscopy [74▪▪] have provided new insights and avenues of research.

Definition of Env signatures that promote bnAb activity has a high potential. Studying a large cohort of transmission pairs defined by genetic linkage of the infecting virus ($N = 303$ pairs), we recently showed that transmitted viruses install a highly similar antibody response in the recipient with certain viruses harboring the capacity to evoke a bnAb response in both partners [60▪▪]. Identification and characterization of virus strains that harbor a bnAb-imprinting capacity are thus key to decipher genetic, structural, and phenotypic features that are linked to the unique immunogenicity of these bnAb-imprinting virus strains.

INFLUENCE OF THE IMMUNE ENVIRONMENT

bnAbs frequently harbor unique features such as poly or autoreactivity, long HCDR3s, extraordinary frequencies of V(D)J mutations, and framework mutations [75–77] underlining that their evolution needs to overcome host tolerance controls. Hence, influencing these control mechanisms may foster the generation of bnAbs in the context of vaccines [78–80]. Evidence that bnAb evolution coincides with a number of distinct immune conditions and factors is increasing. Distinguishing amongst these surrogate markers from true correlates will be an important task for forthcoming studies.

A link between development of breadth and poly/autoreactivity of antibodies has been noted for long [81–86]. In line with an immune

environment that promotes polyreactivity, some studies observed a higher frequency of serum autoantibodies in individuals that mounted a bnAb response [86,87]. As poly/autoreactive antibodies are normally deselected during development [75,76,80], bnAb evolution may thus benefit from deficiencies in immune tolerance controls. Low levels of T-regulatory cells, possibly enabling survival of B-cell intermediates with potential for autoreactivity, have been linked with neutralization breadth [86]. A decline in overall $CD4^+$ cell numbers, which could also reflect a loss in T-regulatory cells, was suggested by some studies to be linked with broad neutralization [32,34,37,59]. Although conceptually very plausible, these data need to be interpreted cautiously. A link between bnAb evolution and $CD4^+$ T-cell counts was not universally observed [29,31,35,36,40]. As viral load is a strong driver of bnAb activity and an inverse association between $CD4^+$ T-cell counts and viral load [88] exists, dissection of confounding factors is essential but not all studies may have the size with statistical power to disentangle effects (Fig. 2).

Thus, this need to dissect confounding influences does not only apply to the specific case of viral load and $CD4^+$ levels. In general, a validation of proposed markers in larger cohorts is an urgent next step in moving forward with the definition of bnAb determinants (see key points).

Several factors implicated in germinal center reaction have been defined as critical for bnAb evolution. Higher levels of T-follicular helper (Tfh) $CD4^+$ cells early in infection have been observed in bnAb inducers [79,86,91,94,95]. Elevated plasma levels of CXCL13, a cytokine involved in B-cell migration to the germinal center, and increased expression of activation-induced deaminase, an enzyme crucial for antibody diversity, have been associated with neutralization breadth [45,91–93,96▪▪] (see Graff-Dubois *et al.*, in this issue). Of note, higher frequency of functional HIV-specific Tfh cells alongside a generally more tolerant immune environment in early life have been suggested as possible causes of the higher frequency of bnAb evolution observed in pediatric HIV infection [97–101].

What prompts changes in immune environment that favor bnAb evolution is not clear. HIV-1 infection causes widespread perturbations of immune cells including B cells [102,103]. Recently, impaired natural killer (NK) cell function and RAB11FIP5 overexpression, which modulates NK function, have been shown to be associated with bnAb development in a cohort of 47 bnAb inducers and 46 matched controls [90▪▪]. The effect was attributed to a diminished ability of NK cells to reduce $CD4^+$ Tfh numbers, which positively impacts B-cell responses [90▪▪].

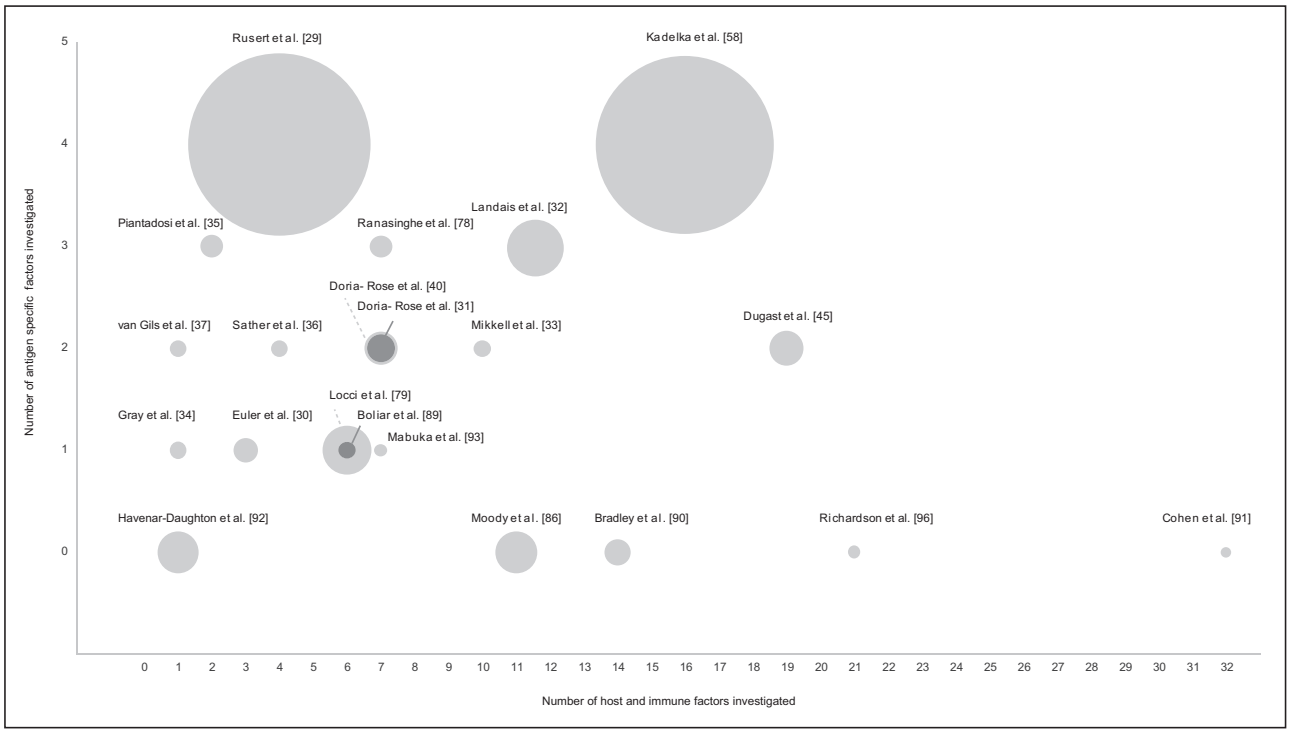

FIGURE 2. Overview of conducted studies [29–37,40,45,58▪▪,78,79,86,89–93], their cohort size, and numbers of variables tested. Bubble area is proportional to cohort size.

Some bnAb specificities are restricted to a limited set of Ig heavy chain germ-line alleles [104]. Although thus far no overall difference in the immunoglobulin gene repertoire of bnAb inducers and nonneutralizers has been observed [105], other genetic determinants may exist that steer bnAb evolution. Specific human leucocyte antigen alleles and variants have been associated with bnAb activity in some cohorts [31,32,86,106,107]. In support of a genetic influence, we recently reported that individuals with black ethnicity more frequently induce bnAb activity [29] and show enhanced anti-Env-binding immunglobulin G1 (IgG1) responses [58▪▪] compared with white study participants of the Swiss 4.5K Study. As anti-Env IgG1 responses were generally elevated amongst bnAb inducers compared with nonneutralizers, a link between an IgG1-driven immune environment and bnAb evolution seems to exist [58▪▪].

System serology studies have revealed multiple aspects of the antibody response repertoire in vaccine recipients, HIV-1-infected nonneutralizers, and bnAb inducers that highlight the differential regulation of antibody responses in the diverse settings [45,58▪▪,96▪▪,108–110]. Titer [32] and avidity [36] of immunglobulin G-binding responses to Env-based antigens can correlate with neutralization breadth. Polyfunctional Fc effector profiles of anti-Env responses are a component of viral control in natural HIV-1 infection [111–113] and distinguish responses to different vaccine regimes [108–110]. Importantly, HIV-specific Fc effector function early in infection was also shown to predict the development of bnAbs [96▪▪] (see Ackerman *et al.*, in this issue). Thus, steering the immune response toward certain Ig subclasses and/or Fc modifications may favor bnAb evolution [58▪▪,96▪▪,108–110].

CONCLUSION

Considering the wealth of factors implicated with bnAb evolution, research efforts need to move into a next phase. Key points of future research (see key points) need to be addressed. It will be critical to dissect factors which are independent drivers of bnAb induction from those that are surrogate markers. This knowledge will aid vaccine development immensely, allowing definition of factors with relevance for immunization regimens. Identification of surrogate markers that allow to reliably predict bnAb evolution is similarly essential as these could provide important tools for early assessment of vaccine efficacy. Many of the data that we currently rely on are based on cohorts of a few dozen to a few hundred individuals (Fig. 2; see also [38]). Although these studies were instrumental in making the initial discoveries, a critical next step will be the validation of the implicated factors in larger cohorts that have the statistical power to reliably disentangle confounding effects.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
- ▪▪ of outstanding interest

1. Plotkin SA. Correlates of protection induced by vaccination. Clin Vacc Immunol 2010; 17:1055–1065.
2. Wei X, Decker JM, Wang S, *et al.* Antibody neutralization and escape by HIV-1. Nature 2003; 422:307–312.
3. Bar KJ, Tsao CY, Iyer SS, *et al.* Early low-titer neutralizing antibodies impede HIV-1 replication and select for virus escape. PLoS Pathog 2012; 8:e1002721.
4. Andrabi R, Bhiman JN, Burton DR. Strategies for a multistage neutralizing antibody-based HIV vaccine. Curr Opin Immunol 2018; 53:143–151.
5. Burton DR, Hangartner L. Broadly neutralizing antibodies to HIV and their role in vaccine design. Annu Rev Immunol 2016; 34:635–659.
6. Escolano A, Dosenovic P, Nussenzweig MC. Progress toward active or passive HIV-1 vaccination. J Exp Med 2017; 214:3–16.
7. Perreau M, Banga R, Pantaleo G. Targeted immune interventions for an HIV-1 cure. Trends Mol Med 2017; 23:945–961.
8. Pegu A, Hessell AJ, Mascola JR, Haigwood NL. Use of broadly neutralizing antibodies for HIV-1 prevention. Immunol Rev 2017; 275:296–312.
9. Burton DR, Mascola JR. Antibody responses to envelope glycoproteins in HIV-1 infection. Nature Immunol 2015; 16:571–576.
10. Trkola A, Kuster H, Rusert P, *et al.* Delay of HIV-1 rebound after cessation of antiretroviral therapy through passive transfer of human neutralizing antibodies. Nat Med 2005; 11:615–622.
11. Mendoza P, Gruell H, Nogueira L, *et al.* Combination therapy with anti-HIV-1 antibodies maintains viral suppression. Nature 2018; 561:479–484.
12. Scheid JF, Horwitz JA, Bar-On Y, *et al.* HIV-1 antibody 3BNC117 suppresses viral rebound in humans during treatment interruption. Nature 2016; 535:556–560.
13. Bar-On Y, Gruell H, Schoofs T, *et al.* Safety and antiviral activity of combination HIV-1 broadly neutralizing antibodies in viremic individuals. Nat Med 2018; 24:1701–1707.
14. Bar KJ, Sneller MC, Harrison LJ, *et al.* Effect of HIV antibody VRC01 on viral rebound after treatment interruption. N Engl J Med 2016; 375:2037–2050.
15. Caskey M, Klein F, Lorenzi JC, *et al.* Viraemia suppressed in HIV-1-infected humans by broadly neutralizing antibody 3BNC117. Nature 2015; 522:487–491.
16. Lynch RM, Boritz E, Coates EE, *et al.* Virologic effects of broadly neutralizing antibody VRC01 administration during chronic HIV-1 infection. Sci Transl Med 2015; 7:319ra206.
17. Borducchi EN, Liu J, Nkolola JP, *et al.* Antibody and TLR7 agonist delay viral rebound in SHIV-infected monkeys. Nature 2018; 563:360–364.
18. Steinhardt JJ, Guenaga J, Turner HL, *et al.* Rational design of a trispecific antibody targeting the HIV-1 Env with elevated antiviral activity. Nat Commun 2018; 9:877.
19. Padte NN, Yu J, Huang Y, Ho DD. Engineering multispecific antibodies against HIV-1. Retrovirology 2018; 15:60.
20. Gruell H, Klein F. Antibody-mediated prevention and treatment of HIV-1 infection. Retrovirology 2018; 15:73.
21. Baba TW, Liska V, Hofmann-Lehmann R, *et al.* Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian-human immunodeficiency virus infection. Nat Med 2000; 6:200–206.

22. Hessell AJ, Rakasz EG, Poignard P, *et al.* Broadly neutralizing human anti-HIV antibody 2G12 is effective in protection against mucosal SHIV challenge even at low serum neutralizing titers. PLoS Pathog 2009; 5:e1000433.
23. Mascola JR, Lewis MG, Stiegler G, *et al.* Protection of Macaques against pathogenic simian/human immunodeficiency virus 89.6PD by passive transfer of neutralizing antibodies. J Virol 1999; 73:4009–4018.
24. Moldt B, Rakasz EG, Schultz N, *et al.* Highly potent HIV-specific antibody neutralization in vitro translates into effective protection against mucosal SHIV challenge in vivo. Proc Natl Acad Sci USA 2012; 109:18921–18925.
25. Parren PW, Marx PA, Hessell AJ, *et al.* Antibody protects macaques against vaginal challenge with a pathogenic R5 simian/human immunodeficiency virus at serum levels giving complete neutralization in vitro. J Virol 2001; 75:8340–8347.
26. Bradley T, Fera D, Bhiman J, *et al.* Structural constraints of vaccine-induced tier-2 autologous HIV neutralizing antibodies targeting the receptor-binding site. Cell Rep 2016; 14:43–54.
27. Sanders RW, van Gils MJ, Derking R, *et al.* HIV-1 VACCINES. HIV-1 neutralizing antibodies induced by native-like envelope trimers. Science 2015; 349:aac4223.
28. Gilbert PB, Juraska M, deCamp AC, *et al.* Basis and Statistical Design of the Passive HIV-1 Antibody Mediated Prevention (AMP) Test-of-Concept Efficacy Trials. Stat Commun Infect Dis 2017; 9:20160001. doi: 10.1515/scid-2016-0001.
29. Rusert P, Kouyos RD, Kadelka C, *et al.* Determinants of HIV-1 broadly neutralizing antibody induction. Nat Med 2016; 22:1260–1267.
30. Euler Z, van Gils MJ, Bunnik EM, *et al.* Cross-reactive neutralizing humoral immunity does not protect from HIV type 1 disease progression. J Infect Dis 2010; 201:1045–1053.
31. Doria-Rose NA, Klein RM, Daniels MG, *et al.* Breadth of human immunodeficiency virus-specific neutralizing activity in sera: clustering analysis and association with clinical variables. J Virol 2010; 84:1631–1636.
32. Landais E, Huang X, Havenar-Daughton C, *et al.* Broadly neutralizing antibody responses in a large longitudinal sub-Saharan HIV primary infection cohort. PLoS Pathog 2016; 12:e1005369.
33. Mikell I, Sather DN, Kalams SA, *et al.* Characteristics of the earliest cross-neutralizing antibody response to HIV-1. PLoS Pathog 2011; 7:e1001251.
34. Gray ES, Madiga MC, Hermanus T, *et al.* The neutralization breadth of HIV-1 develops incrementally over four years and is associated with CD4+ T cell decline and high viral load during acute infection. J Virol 2011; 85:4828–4840.
35. Piantadosi A, Panteleeff D, Blish CA, *et al.* Breadth of neutralizing antibody response to human immunodeficiency virus type 1 is affected by factors early in infection but does not influence disease progression. J Virol 2009; 83:10269–10274.
36. Sather DN, Armann J, Ching LK, *et al.* Factors associated with the development of cross-reactive neutralizing antibodies during human immunodeficiency virus type 1 infection. J Virol 2009; 83:757–769.
37. van Gils MJ, Euler Z, Schweighardt B, *et al.* Prevalence of cross-reactive HIV-1-neutralizing activity in HIV-1-infected patients with rapid or slow disease progression. AIDS 2009; 23:2405–2414.
38. Subbaraman H, Schanz M, Trkola A. Broadly neutralizing antibodies: What is needed to move from a rare event in HIV-1 infection to vaccine efficacy? Retrovirology 2018; 15:52.
39. Moore PL, Williamson C, Morris L. Virological features associated with the development of broadly neutralizing antibodies to HIV-1. Trends Microbiol 2015; 23:204–211.
40. Doria-Rose NA, Klein RM, Manion MM, *et al.* Frequency and phenotype of human immunodeficiency virus envelope-specific B cells from patients with broadly cross-neutralizing antibodies. J Virol 2009; 83:188–199.
41. Pereyra F, Addo MM, Kaufmann DE, *et al.* Genetic and immunologic heterogeneity among persons who control HIV infection in the absence of therapy. J Infect Dis 2008; 197:563–571.
42. Euler Z, van den Kerkhof TL, van Gils MJ, *et al.* Longitudinal analysis of early HIV-1-specific neutralizing activity in an elite neutralizer and in five patients who developed cross-reactive neutralizing activity. J Virol 2012; 86:2045–2055.
43. Freund NT, Wang H, Scharf L, *et al.* Coexistence of potent HIV-1 broadly neutralizing antibodies and antibody-sensitive viruses in a viremic controller. Sci Transl Med 2017; 9: eaal2144. doi: 10.1126/scitranslmed.aal2144.
44. Scheid JF, Mouquet H, Feldhahn N, *et al.* Broad diversity of neutralizing antibodies isolated from memory B cells in HIV-infected individuals. Nature 2009; 458:636–640.
45. Dugast AS, Arnold K, Lofano G, *et al.* Virus-driven inflammation is associated with the development of bNAbs in spontaneous controllers of HIV. Clin Infect Dis 2017; 64:1098–1104.
46. Lynch RM, Shen T, Gnanakaran S, Derdeyn CA. Appreciating HIV type 1 diversity: subtype differences in Env. AIDS Res Hum Retrovirus 2009; 25:237–248.
47. Wibmer CK, Bhiman JN, Gray ES, *et al.* Viral escape from HIV-1 neutralizing antibodies drives increased plasma neutralization breadth through sequential recognition of multiple epitopes and immunotypes. PLoS Pathog 2013; 9:e1003738.
48. Moore PL, Gray ES, Wibmer CK, *et al.* Evolution of an HIV glycan-dependent broadly neutralizing antibody epitope through immune escape. Nat Med 2012; 18:1688–1692.
49. Doria-Rose NA, Schramm CA, Gorman J, *et al.* Developmental pathway for potent V1V2-directed HIV-neutralizing antibodies. Nature 2014; 509:55–62.
50. ■■ Williams KL, Wang B, Arenz D, *et al.* Superinfection drives HIV neutralizing antibody responses from several b cell lineages that contribute to a polyclonal repertoire. Cell Rep 2018; 23:682–691.

Study highlights the effect of superinfection on diversification of the neutralizing antibody response.

51. Moore PL, Sheward D, Nonyane M, *et al.* Multiple pathways of escape from HIV broadly cross-neutralizing V2-dependent antibodies. J Virol 2013; 87:4882–4894.
52. Cortez V, Odem-Davis K, McClelland RS, *et al.* HIV-1 superinfection in women broadens and strengthens the neutralizing antibody response. PLoS Pathog 2012; 8:e1002611.
53. Powell RL, Kinge T, Nyambi PN. Infection by discordant strains of HIV-1 markedly enhances the neutralizing antibody response against heterologous virus. J Virol 2010; 84:9415–9426.
54. ■■ Sheward DJ, Marais J, Bekker V, *et al.* HIV superinfection drives de novo antibody responses and not neutralization breadth. Cell Host Microbe 2018; 24:593–599.e3.

Study shows that superinfection may predominantly generate novel neutralizing antibody responses but not bnAb activity.

55. Courtney CR, Mayr L, Nanfack AJ, *et al.* Contrasting antibody responses to intrasubtype superinfection with CRF02_AG. PLoS One 2017; 12:e0173705.
56. Wagner GA, Landais E, Caballero G, *et al.* Intrasubtype B HIV-1 Superinfection Correlates with Delayed Neutralizing Antibody Response. J Virol 2017; 91: e00475-17; doi: 10.1128/JVI.00475-17.
57. Cortez V, Wang B, Dingens A, *et al.* The broad neutralizing antibody responses after HIV-1 superinfection are not dominated by antibodies directed to epitopes common in single infection. PLoS Pathog 2015; 11:e1004973.
58. ■■ Kadelka C, Liechti T, Ebner H, *et al.* Distinct, IgG1-driven antibody response landscapes demarcate individuals with broadly HIV-1 neutralizing activity. J Exp Med 2018; 215:1589–1608.

Population-wide survey of HIV-1 binding antibody responses defines factors associated with bnAb development.

59. Lynch RM, Tran L, Louder MK, *et al.* The development of CD4 binding site antibodies during HIV-1 infection. J Virol 2012; 86:7588–7595.
60. ■■ Kouyos RD, Rusert P, Kadelka C, *et al.* Tracing HIV-1 strains hat imprint broadly neutralizing antibody responses. Nature 2018; 561:406–410.

Based on neutralizing and binding plasma antibody analysis of a large transmission cohort, the study introduces the concept of bnAb imprinters, Env variants that have the capacity to install a similar bnAb response.

61. ■ Mabvakure BM, Scheepers C, Garrett N, *et al.* Positive selection at key residues in the HIV envelope distinguishes broad and strain-specific plasma neutralizing antibodies. J Virol 2019; 93:e01685-18.

Study highlights the impact of specific Env signatures on breadth development in individual bnAb donors.

62. Smith SA, Burton SL, Kilembe W, *et al.* Diversification in the HIV-1 envelope hyper-variable domains V2, V4, and V5 and higher probability of transmitted/founder envelope glycosylation favor the development of heterologous neutralization breadth. PLoS Pathog 2016; 12:e1005989.
63. Rademeyer C, Moore PL, Taylor N, *et al.* Genetic characteristics of HIV-1 subtype C envelopes inducing cross-neutralizing antibodies. Virology 2007; 368:172–181.
64. van den Kerkhof TL, Feenstra KA, Euler Z, *et al.* HIV-1 envelope glycoprotein signatures that correlate with the development of cross-reactive neutralizing activity. Retrovirology 2013; 10:102.
65. Gnanakaran S, Daniels MG, Bhattacharya T, *et al.* Genetic signatures in the envelope glycoproteins of HIV-1 that associate with broadly neutralizing antibodies. PLoS Comput Biol 2010; 6:e1000955.
66. Rawi R, Mall R, Shen C-H, *et al.* Accurate prediction of antibody resistance in clinical HIV-1 isolates. bioRxiv 2018; 364828. doi: https://doi.org/10.1101/364828.
67. ■■ Bricault CA, Yusim K, Seaman MS, *et al.* HIV-1 neutralizing antibody signatures and application to epitope-targeted vaccine design. Cell Host Microbe 2019; 25:59–72.e8.

Study presents a strategy to systematically analyze Env signatures linked with bnAb activity.

68. ■ Haddox HK, Dingens AS, Hilton SK, *et al.* Mapping mutational effects along the evolutionary landscape of HIV envelope. eLife 2018; 7:.

Study provides detailed insight into Env evolution.

69. ■■ Dingens AS, Arenz D, Weight H, *et al.* An antigenic atlas of HIV-1 escape from broadly neutralizing antibodies distinguishes functional and structural epitopes. Immunity 2019; 50:520–532.e3.

Study provides detailed Env signature information of known bnAbs at unprecedented level.

70. ■ Otsuka Y, Schmitt K, Quinlan BD, *et al.* Diverse pathways of escape from all well characterized VRC01-class broadly neutralizing HIV-1 antibodies. PLoS Pathog 2018; 14:e1007238.

Study describes novel approach to dissect Env signatures of specific bnAbs.

71. Raju N, Setliff I, Georgiev IS. NFPws: a web server for delineating broadly
■ neutralizing antibody specificities from serum HIV-1 neutralization data. Bioinformatics 2019; btz097, https://doi.org/10.1093/bioinformatics/btz097.

Study describes novel tool for plasma neutralization delineation.

72. Doria-Rose NA, Altae-Tran HR, Roark RS, *et al.* Mapping polyclonal HIV-1 antibody responses via next-generation neutralization fingerprinting. PLoS Pathog 2017; 13:e1006148.

73. Cheng HD, Grimm SK, Gilman MS, *et al.* Fine epitope signature of antibody
■ neutralization breadth at the HIV-1 envelope CD4-binding site. JCI Insight 2018; 3:.

Study describes potential of conventional binding assays in dissecting bnAb epitope signatures.

74. Bianchi M, Turner HL, Nogal B, *et al.* Electron-microscopy-based epitope
■■ mapping defines specificities of polyclonal antibodies elicited during HIV-1 BG505 envelope trimer immunization. Immunity 2018; 49:288–300.e8.

Study introduces a novel plasma epitope mapping strategy on the basis of electron microscopy analysis of plasma antibody binding to Env trimer.

75. Haynes BF, Fleming J, St. Clair EW, *et al.* Cardiolipin polyspecific autoreactivity in two broadly neutralizing HIV-1 antibodies. Science 2005; 308:1906–1908.

76. Haynes BF, Moody MA, Verkoczy L, *et al.* Antibody polyspecificity and neutralization of HIV-1: a hypothesis. Hum Antibodies 2005; 14:59–67.

77. Finney J, Kelsoe G. Poly- and autoreactivity of HIV-1 bNAbs: implications for vaccine design. Retrovirology 2018; 15:53.

78. Ranasinghe S, Soghoian DZ, Lindqvist M, *et al.* HIV-1 antibody neutralization breadth is associated with enhanced HIV-specific CD4+ T cell responses. J Virol 2015; 90:2208–2220.

79. Locci M, Havenar-Daughton C, Landais E, *et al.* Human circulating PD-1+CXCR3-CXCR5+ memory Tfh cells are highly functional and correlate with broadly neutralizing HIV antibody responses. Immunity 2013; 39:758–769.

80. Kelsoe G, Haynes BF. Host controls of HIV broadly neutralizing antibody development. Immunol Rev 2017; 275:79–88.

81. Mouquet H, Scheid JF, Zoller MJ, *et al.* Polyreactivity increases the apparent affinity of anti-HIV antibodies by heteroligation. Nature 2010; 467:591–595.

82. Diskin R, Marcovecchio PM, Bjorkman PJ. Structure of a clade C HIV-1 gp120 bound to CD4 and CD4-induced antibody reveals anti-CD4 polyreactivity. Nat Struct Mol Biol 2010; 17:608–613.

83. Mouquet H, Warncke M, Scheid JF, *et al.* Enhanced HIV-1 neutralization by antibody heteroligation. Proc Natl Acad Sci USA 2012; 109:875–880.

84. Liu MF, Yang G, Wiehe K, *et al.* Polyreactivity and autoreactivity among HIV-1 antibodies. J Virol 2015; 89:784–798.

85. Liao HX, Lynch R, Zhou T, *et al.* Co-evolution of a broadly neutralizing HIV-1 antibody and founder virus. Nature 2013; 496:469–476.

86. Moody MA, Pedroza-Pacheco I, Vandergrift NA, *et al.* Immune perturbations in HIV-1-infected individuals who make broadly neutralizing antibodies. Sci Immunol 2016; 1:aag0851.

87. Gray ES, Taylor N, Wycuff D, *et al.* Antibody specificities associated with neutralization breadth in plasma from human immunodeficiency virus type 1 subtype C-infected blood donors. J Virol 2009; 83:8925–8937.

88. Mellors JW, Munoz A, Giorgi JV, *et al.* Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. Ann Intern Med 1997; 126:946–954.

89. Boliar S, Murphy MK, Tran TC, *et al.* B-lymphocyte dysfunction in chronic HIV-1 infection does not prevent cross-clade neutralization breadth. J Virol 2012; 86:8031–8040.

90. Bradley T, Peppa D, Pedroza-Pacheco I, *et al.* RAB11FIP5 expression and
■■ altered natural killer cell function are associated with induction of HIV broadly neutralizing antibody responses. Cell 2018; 175:387.e17–399.e17.

Study defines novel immune factors, RAB11FIP5 overexpression, and deficiency in NK cell function, to be linked with bnAb activity. A diminished ability of NK cells to reduce CD4 Tfh numbers is suggested to positively reflect on B-cell responses.

91. Cohen K, Altfeld M, Alter G, Stamatatos L. Early preservation of CXCR5+ PD-1+ helper T cells and B cell activation predict the breadth of neutralizing antibody responses in chronic HIV-1 infection. J Virol 2014; 88: 13310–13321.

92. Havenar-Daughton C, Lindqvist M, Heit A, *et al.* CXCL13 is a plasma biomarker of germinal center activity. Proc Natl Acad Sci 2016; 113:2702.

93. Mabuka JM, Dugast AS, Muema DM, *et al.* Plasma CXCL13 but not B cell frequencies in acute HIV infection predicts emergence of cross-neutralizing antibodies. Front Immunol 2017; 8:1104.

94. Yamamoto T, Lynch RM, Gautam R, *et al.* Quality and quantity of TFH cells are critical for broad antibody development in SHIVAD8 infection. Sci Transl Med 2015; 7:298ra120.

95. Moysi E, Petrovas C, Koup RA. The role of follicular helper CD4 T cells in the development of HIV-1 specific broadly neutralizing antibody responses. Retrovirology 2018; 15:54.

96. Richardson SI, Chung AW, Natarajan H, *et al.* HIV-specific Fc effector
■■ function early in infection predicts the development of broadly neutralizing antibodies. PLoS Pathog 2018; 14:e1006987.

Study highlights the importance of events early in infection, particularly Fc effector function, that are linked with bnAb evolution.

97. Roider J, Maehara T, Ngoepe A, *et al.* High-frequency, functional HIV-specific T-follicular helper and regulatory cells are present within germinal centers in children but not adults. Front Immunol 2018; 9:1975.

98. Goo L, Chohan V, Nduati R, Overbaugh J. Early development of broadly neutralizing antibodies in HIV-1-infected infants. Nat Med 2014; 20:655–658.

99. Muenchhoff M, Adland E, Karimanzira O, *et al.* Nonprogressing HIV-infected children share fundamental immunological features of nonpathogenic SIV infection. Sci Transl Med 2016; 8:358ra125.

100. Mold JE, McCune JM. At the crossroads between tolerance and aggression: Revisiting the 'layered immune system' hypothesis. Chimerism 2011; 2:35–41.

101. Kollmann TR, Levy O, Montgomery RR, Goriely S. Innate immune function by Toll-like receptors: distinct responses in newborns and the elderly. Immunity 2012; 37:771–783.

102. Moir S, Fauci AS. B-cell exhaustion in HIV infection: the role of immune activation. Curr Opin HIV AIDS 2014; 9:472–477.

103. Moir S, Chun TW, Fauci AS. Pathogenic mechanisms of HIV disease. Annu Rev Pathol 2011; 6:223–248.

104. Zhou T, Lynch RM, Chen L, *et al.* Structural repertoire of HIV-1-neutralizing antibodies targeting the CD4 supersite in 14 donors. Cell 2015; 161:1280–1292.

105. Scheepers C, Shrestha RK, Lambson BE, *et al.* Ability to develop broadly neutralizing HIV-1 antibodies is not restricted by the germline Ig gene repertoire. J Immunol 2015; 194:4371–4378.

106. Euler Z, van Gils MJ, Boeser-Nunnink BD, *et al.* Genome-wide association study on the development of cross-reactive neutralizing antibodies in HIV-1 infected individuals. PLoS One 2013; 8:e54684.

107. Rouers A, Klingler J, Su B, *et al.* HIV-specific B cell frequency correlates with neutralization breadth in patients naturally controlling HIV-infection. EBioMedicine 2017; 21:158–169.

108. Chung AW, Kumar MP, Arnold KB, *et al.* Dissecting polyclonal vaccine-induced humoral immunity against HIV using systems serology. Cell 2015; 163:988–998.

109. Chung AW, Ghebremichael M, Robinson H, *et al.* Polyfunctional Fc-effector profiles mediated by igg subclass selection distinguish RV144 and VAX003 vaccines. Sci Transl Med 2014; 6:228ra38.

110. Mahan AE, Jennewein MF, Suscovich T, *et al.* Antigen-specific antibody glycosylation is regulated via vaccination. PLoS Pathog 2016; 12:e1005456.

111. Ackerman ME, Mikhailova A, Brown EP, *et al.* Polyfunctional HIV-specific antibody responses are associated with spontaneous HIV control. PLoS Pathog 2016; 12:e1005315.

112. Lai JI, Licht AF, Dugast AS, *et al.* Divergent antibody subclass and specificity profiles but not protective HLA-B alleles are associated with variable antibody effector function among HIV-1 controllers. J Virol 2014; 88:2799–2809.

113. Sadanand S, Das J, Chung AW, *et al.* Temporal variation in HIV-specific IgG subclass antibodies during acute infection differentiates spontaneous controllers from chronic progressors. AIDS 2018; 32:443–450.